ACOUSTIC BLOOD ANALYZER FOR ASSESSING BLOOD PROPERTIES

Introduction

5   This application claims the benefit of priority from
U.S. Provisional Application Serial No. 60/463,557, filed
April 16, 2003, which is herein incorporated by reference in
its entirety.

10 Field of the Invention

An acousto-mechanical blood analyzer, also referred to
herein as an acoustic blood analyzer, is provided with a
point-of-care and self-test clotting sensor that measures
multiple blood properties including, but not limited to,
15   blood coagulation and platelet function. The acousto-
mechanical blood analyzer comprises a transducer section of
one or more acoustic sensors. Preferably the acoustic
sensor or sensor further comprises a bioactive surface or
coating which promotes specific blood-sensor interactions.
20   The analyzer also preferably further comprises a blood
sampling means, a fluidic section to deliver and distribute
a blood sample to the acoustic sensor or sensors, an
electronic section means, which excites the acoustic sensor
or sensors and detects changes in the operational parameters
25   of the transducer section, and a packaging section or
housing which provides mechanical and functional integrity
to the transducer, fluidic, and electronic section means of
the analyzer. This packaging section also provides an
interface for the analyzer with analytical laboratory
30   systems and computer based data processing, storage and
display systems. Methods for using the acoustic blood
analyzer to measure blood properties including, but not
limited to, blood coagulation and platelet function are also
provided. The analyzer and methods of the present invention

are particularly useful in identifying, diagnosing, and monitoring subjects at high risk for blood clots and excessive bleeding.

Background of the Invention

Thrombotic disease is considered to be a major health problem affecting millions of patients in the United States alone and its manifestations cause significant morbidity and mortality. Estimates of incidence vary because many of the events go unnoticed, including the fatal ones. In particular, as many as 2,000,000 Americans each year suffer from deep vein thrombosis (clots) in one form or another. Approximately half of these incidences occur in surgical patients or individuals with cancer, other illnesses or trauma while the other half occurs for no known reasons. Surgical procedures in general predispose patients to risk of developing blood clots that may result in life-threatening clinical conditions, including deep vein thrombosis (DVT), pulmonary embolism (PE) or stroke. Procedures in which such risks are especially high include cardio-pulmonary bypass surgery (CPB), coronary artery bypass grafting (CABG), gynecological procedures, neurosurgery, orthopedic surgery, percutaneous transluminal coronary angioplasty (PTCA), and posttrauma reconstructive surgery (Geerts, W.H. Semin Thromb. Hemost. 1996 22(2):19-24; Poller, L. and Hirsh, J. "Optimal Therapeutic Ranges for Oral Anticoagulation," in *Thrombosis in Cardiovascular Disorders*, Fuster V, Verstraete M (eds), Philadelphia, Saunders, pp 161-173, 1992; Ogilby et al. Cath Cardio Diag, 1989 18:206-209; Narins et al. Circulation, 1996 93:667-671). The risk of clotting is relatively high for these operations because they are inherently more invasive and expose the patient's blood to foreign surfaces that can initiate coagulation. To minimize the risks associated with

such surgical procedures, patients are placed on anticoagulants (blood thinners). Without appropriate prophylaxis, it is estimated that thrombotic complications would occur in as many as 50% of patients undergoing

5   orthopedic surgery and as many as 25% of patients undergoing other surgeries.

Individuals at particularly high risk for developing blood clots are those who have had them in the past. Clots form most commonly in the legs, but many (>50%) travel to

10   the lungs, resulting in pulmonary embolism (which can be fatal).

New antithrombins and antiplatelet agents are now available to better manage these complex conditions and more efficiently control the thrombotic process. A new generation

15   of oral anticoagulants is also being used extensively in outpatient settings. In the United States, more than 1.5 million people with atrial fibrillation or mechanical heart valves are currently taking some kind of oral anticoagulant to help prevent stroke. A resulting complication of these

20   treatments, however, is often bleeding, which can be severe. As a result, patient monitoring is critical to ensure appropriate treatment regimens. To ensure that surgical patients receive the correct dose of anticoagulant and antithrombotic agents, each patient's hemostatic status/

25   ranking before, during, and after the surgical procedure must be assessed. Importantly, for patients who are placed on long-term oral anticoagulant therapy, additional postdischarge monitoring is of particular value (Hirsh J, Salzman EW, Marder VJ, "Treatment of Thromboembolism," in

30   *Hemostasis and Thrombosis: Basic Principles and Clinical Practices* (3rd ed), Colman RC, Hirsh J, Marder VJ, et al. (eds), Philadelphia, Lippincott, pp 1346-1366, 1994).

The traditional coagulation tests have been available for decades. The majority of such tests are functional end-point assays, in which a patient sample (plasma or whole blood) is incubated with exogenous reagents that activate the

5   coagulation cascade, and the time to clot formation is measured. The clotting time of the patient sample is then compared to the clotting time of pooled normal plasma or whole blood to provide a standard measurement of the patient's hemostatic status. These clotting assays are

10 commonly used as screening tests that evaluate the functioning of both the patient's intrinsic and extrinsic coagulation systems (Carville, D.G.M. and Guyer, K.E. IVD Tech 1998 4(4):59-66).

The Activated Partial Thromboplastin Time Test (APTT)

15 is used to evaluate the intrinsic coagulation pathway, which includes factors I, II, V, VIII, IX, X, XI, and XII. The test is performed using a plasma sample, in which the intrinsic pathway is activated by the addition of phospholipid, an activator (ellagic acid, kaolin, or

20 micronized silica), and Ca^{2+}. Formation of the Xase and prothrombinase complexes on the surface of the phospholipid enables prothrombin to be converted into thrombin, with subsequent clot formation. The result of the APTT test is the time (in seconds) required for this reaction. APTT is

25 used to assess the overall competence of a patient's coagulation system, as a preoperative screening test for bleeding tendencies, and as a routine test for monitoring heparin therapy.

The Activated Clotting Time Test (ACT) is a screening

30 test that resembles the activated partial thromboplastin time (APTT) test, but is performed using fresh whole blood samples. ACT is used primarily to monitor a patient's coagulation status in connection with "invasive" procedures

that involve the administration of high doses of heparin (e.g., CPB and PTCA). It is important to monitor a patient's response to heparin during such procedures because under dosing can result in pathological thrombus formation,

5   whereas overdosing can lead to serious hemorrhagic complications.

The Prothrombin Time Test (PT) was first described in the mid 1930s. The PT test measures the tissue factor-induced coagulation time of blood or plasma. It is used as a

10   screening test to evaluate the integrity of the extrinsic coagulation pathway, and is sensitive to coagulation factors I, II, V, VII, and X. The test is performed by adding thromboplastin and Ca^{2+} to a patient sample and measuring the time for clot formation. A prolonged clotting time suggests

15   the presence of an inhibitor to, or a deficiency in, one or more of the coagulation factors of the extrinsic pathway. But PT clotting time can also be prolonged for patients on warfarin therapy, or for those with vitamin K deficiency or liver dysfunction. The PT test can provide an assessment of

20   the extrinsic coagulation pathway, and is widely used to monitor oral anticoagulation therapy.

The Thrombin Clotting Time Test (TCT) measures the rate of a patient's clot formation compared to that of a normal plasma control. The test is performed by adding a standard

25   amount of thrombin to a patient's plasma that has been depleted of platelets, and measuring the time required for a clot to form. This test has been used as an aid in the diagnosis of disseminated intravascular coagulation (DIC) and liver disease.

30   These traditional coagulation tests are all generally performed in a central laboratory, and not in near-patient settings or by self-testing.

Beyond the commonly used test for screening, there are a number of more complex tests that may be used in the diagnosis of a patient's coagulative status. The assays enable clinicians to reduce the number of possible explanations for a prolonged clotting time found in screening assays such as APTT, PT, and TCT.

One such test is a clotting assay for factor VIIa, which has found utility in monitoring patients with severe factor IX deficiency. The level of factor VIIa in these patients has been reported to be less than 10% of the level found in healthy control subjects.

An assay for factor VIII is useful as a diagnostic test for classical hemophilia.

An assay is also available for measuring the level of the activation peptide factor IXa or the factor IXa-antithrombin III complex. These measurements are used to determine the levels of factor IXa or factor VII-tissue mediated complex. Patients with congenital deficiencies of factor VII may be monitored with this test.

Assays for activated protein C resistance, antithrombin, protein C deficiency, and protein S deficiency are also available. Asymptomatic individuals who have heterogeneous deficiencies of proteins C and S, and resistance to activated protein C, have significantly elevated levels of the prothrombin fragment F1.2 compared to controls. More recently, immunochemical assays have been developed that characterize a patient's hemostatic status by determining the concentration of peptides, proteins, and factors of the coagulation cascade found in the patient's sample.

In addition to the factors of the coagulation cascade, platelets play an extremely important role in hemostasis. There is a growing number of antiplatelet agents that are

indicated for use in conjunction with anticoagulants. Some of the more common antiplatelet agents include aspirin (a weak antiplatelet agent that affects cyclooxygenase activity during the life of the platelet), clopidogrel and

5   ticlopidine (inhibitors of ADP-mediated activation), and various glycoprotein IIb/IIIa (GPIIb/IIIa) antagonists.

These agents are now recognized as having an extremely important role in the prevention of thromboembolic processes, and the most effective medicines, the GPIIb/IIIa

10 inhibitors, require careful dosing and monitoring to avoid the adverse side effect of hemorrhagic (bleeding) complications. Thus, monitoring of antiplatelet therapy is also of major interest in most clinical settings where invasive procedures are performed.

15 In addition to clotting time assays which measure the dynamics of various parts of the coagulation cascade, several assays of the physical properties of the clot, which indicate the combined effects of platelets, fibrin, in concert the coagulation cascade, are available and often

20 used to manage patients in cardiovascular surgery and other critical arenas. The TEG® Coagulation Analyzer monitors the thrombodynamic properties of blood as it is induced to clot under a low shear environment resembling sluggish venous flow. The patterns of changes in shear elasticity enable the

25 determination of the kinetics of clot formation and growth as well as the strength and stability of the formed clot. The strength and stability of the clot provide information about the ability of the clot to perform the work of hemostasis, while the kinetics determine the adequacy of

30 qualitative factors available to clot formation. Other physical clotting assays such as the clot rheometer and the sonoclot analyzer have been developed. At this time, physical clotting assays indicate some platelet-related

hemostatic deficiencies, but cannot directly assay platelet aggregation or adhesion.

For the majority of patients, laboratory monitoring may not be feasible for a variety of practical reasons.

5   Consequently, near-patient ("self-test") hemostasis testing technologies have to provide rapid results in both the clinical and in the outpatient settings. These technologies when fully developed will make significant impact on the choice of therapeutic interventions, patient follow-up and

10   management strategy.

Advances made in microfabrication technologies, inexpensive signal processing systems, and the progress in design of biological sensing interfaces have made possible the development of a variety of new biosensors. One of the

15   emerging applications in the delivery of health care is what is becoming known as near-patient or personalized medicine. For example, there is an increasing use of personal monitoring devices such as glucose sensors for diabetics and for HIV detection.

20   Near-patient (self administered) tests using novel analyzers that boast microvolume test capabilities for blood coagulation have been described. Such tests have been promoted for use in both acute areas of the hospital and by outpatients (e.g., on warfarin therapy) who do not have easy

25   access to a central coagulation laboratory. Patients must be specifically trained, however, to perform the currently available prothrombin time (PT) self-testing at home. Accordingly, the suitability and accuracy of such advanced technologies for assessing the adequacy of a patient's

30   hemostatic system remains a concerns because of the potential for active surface-to-volume effects when small samples are employed, the complexity of the medium being

tested (i.e., blood), and the effects that sample processing can have on both the coagulation and thrombotic pathways.

A number of patents describe coagulation measurement using a variety of methods and devices.

5   For example, U.S. Patent 5,110,727 and 4,849,340, disclose a commercial point-of-care system referred to as TAS (Thrombolytic Assessment System). The TAS system uses paramagnetic iron oxide particles (PIOP)/dry chemistry technology. It is based on near-infrared sensing of the

10   motion of PIOP contained in a dry reagent situated as a film on the surface of a flat-capillary reaction chamber mounted on a plastic test card. The PIOP are subjected to an oscillating magnetic field generated by the instrument in which the test card is placed. When blood or plasma is added

15   to a sample well of the test card, the sample enters the reaction chamber, reconstituting the reagent and freeing the PIOP so that they can move in response to changes in the magnetic field with time. The PIOP motion changes when an *in vitro* thrombus forms. This change results from PIOP

20   entrapment during fibrin polymerization or release during fibrinolysis, providing a kinetic response curve from which the analyzer determines clotting time and a parameter characterizing fibrinolysis process. The TAS system is used commercially in several products.

25   U.S. Patent 3,695,842 describes a precision magnet in a reagent-containing test tube for measurement of clot formation. When the test tube is filled with sample and inserted in a test well containing a magnetic detector, the tube slowly rotates. When the clot begins to form, a change

30   in the position of the magnet is detected.

U.S. Patent 5,372,946 discloses a disposable cuvette within which is formed a capillary conduit having at least one restricted region for measurement of clot formation. In

this device, blood is forced to flow through the restricted region back and forth within a test channel. Two photo-optical detectors are used to measure the speed of sample movement. This patent sets forth the basis for the ProTime

5 microcoagulation system.

U.S. Patent 4,756,884 discloses a technology developed by Biotrack based on optical measurement of a speckle pattern from cells or particles from a sample illuminated by coherent light and flowing in a long capillary track of a

10 plastic reagent-containing cartridge. When clotting occurs, the speckle pattern measurement indicates cessation of flow in the capillary track.

U.S. Patent 4,599,219 discloses a cylindrical plastic cartridge with a plunger assembly terminating in a "flag" at

15 one end and a "daisy" at the other end. The plunger, situated in a reaction chamber above a reagent chamber, is moved by an external mechanical actuator. Flag movement through the clot reaction chamber is timed by a photo-optical detector, the end point being established when

20 fibrin forms on the daisy and slows the plunger movement. This patent sets forth the basis for the Medtronic ACT-II and HepconHMS coagulation assays.

U.S. Patent 5,167,145 describes a technology for measuring clot formation, which uses infrared

25 electromagnetic energy. Infrared electromagnetic transmission changes through a sample from a source of infrared energy to suitable detection electronics producing a peak signal representing the clotting time.

U.S. Patent 5,601,995 discloses a method where a blood

30 sample is applied to a porous sheet and at least one of a spreading extent and a spreading rate are measured by either an optical property, or an electrical potential across the

porous sheet to determine the propensity of the sample to coagulate.

U.S. Patent 5,418,143 discloses a method for detecting clot formation in a whole blood sample using a test strip
5   comprising a porous membrane having a coagulation initiator and a substrate impregnated therein. The substrate is activated by thrombin, and produces a detectable fluorescent signal as the output.

U.S. Patent 6,200,532 discloses several technologies
10   applied to point-of-care blood clot measurement, based on motion of a magnetically excited "bender" which deforms the blood sample. The bender motion is detected by piezoelectric or optical means. This patent also describes sample handling, reagent mixing, packaging, and the use of
15   microporous membranes to separate plasma from blood cells for conducting plasma assays. Use of a piezoelectric transducer to measure changes to an electromagnetic wave in the coagulating blood sample is also described.

However, these above-described methods and devices have
20   disadvantages in their complexity resulting from the use of two or more distinct mechanisms to probe or perturb the clotting blood, and to measure the response of the clot. Further, they lack specificity, in that they can only measure coagulation or platelet aggregation in bulk and/or
25   in response to bulk stimulants. In addition, many of these devices do not differentiate between clots of differing mechanical properties, and those methods that do (e.g. TEG) are expensive, cumbersome and require specialized training and large blood sample volumes.

30   Thus, there is a need for better near-patient /self-test assays capable of accurate and timely platelet-function testing as well as analysis of the coagulation status of blood. The optimal near-patient/self test coagulation

analyzer will be of small size and light weight for
portability, capable of achieving physiological temperatures
(37°C), and easy to use and requiring no specialized
training. This analyzer is preferably capable of producing
5   rapid quantitative results to local as well as remote sites
using reagents that have been standardized to provide
international normalized ratios. The analyzer must require
only small sample sizes of whole blood. Preferably,
components of the analyzer contacting the patient sample are
10   disposable and the entire equipment and operating costs for
the analyzer are relatively low.

Summary of the Invention

An object of the present invention is to provide an
15   acoustic blood analyzer with a point-of-care and self-test
clotting sensor that measures blood properties including,
but in no way limited to, coagulation and platelet function.
In simplest form, the acoustic blood analyzer comprises a
transducer section of one or more acoustic sensors,
20   preferably piezoelectric, electrostrictive,
magnetostrictive, acoustooptic or thermo(piro) acoustic
biosensors or a combination thereof, more preferably
piezoelectric biosensors. In a preferred embodiment
acoustic sensors of the transducer section further comprise
25   a bioactive surface or coating which promotes specific
blood-sensor interactions. Also preferred is that the
acoustic blood analyzer further comprise a blood sampling
means to obtain a blood sample, a fluidic section to deliver
and distribute a blood sample to the acoustic sensor or
30   sensors; an electronic section means which excites the
sensor or sensors and detects changes in the operational
parameters of the transducer section; and a packaging
section or housing which provides mechanical and functional

integrity to the transducer, fluidic and electronic section
means of the analyzer as well as an interface for the
analyzer with analytical laboratory systems and computer
based data processing, storage and display systems.

5   Another object of the present invention is to provide a
method for measuring blood properties including, but in no
way limited to, coagulation and platelet function in a blood
sample of a subject which comprises analyzing the blood
sample in this acoustic blood analyzer. Qualitative as well
10   as quantitative blood properties can be measured with the
acoustic blood analyzer of the present invention. Further,
use of the acoustic blood analyzer allows for relationships
between the acoustic sensor electrical parameters and blood
properties and their time characteristics to be identified.

15   Another object of the present invention is to provide a
method for identifying, diagnosing and monitoring subjects
at high risk for blood clots and excessive bleeding which
comprises obtaining a blood sample from a subject and
analyzing the blood sample in this acoustic blood analyzer.
20   For purposes of monitoring subjects, sampling and analysis
may be performed on a periodic basis depending upon the
condition and the severity of the condition in the subject.

Yet another object of the present invention is to
provide a method of using different vibrational modes of
25   acoustic sensors including, but not limited to shear,
torsional, and compressional modes and combinations thereof
to characterize blood properties.

Brief Description of the Figures
30   Figures 1A through 1D provide data from the 3rd harmonic
of a 5 MHz sensor, penetration depth approximately 150 nm.

Figure 1A shows a shear wave sensor schematic.

Figure 1B shows the change in attenuation (Δα) during platelet adhesion (1 – 5 minutes) and clotting (5 – 25 minutes for "Clot" trace).

Figure 1C shows the dynamics of attenuation change are delayed when clotting is prolonged.

Figure 1D is a linegraph wherein showing the Δα vs. time slope to be zero when time is equal to clotting time (time/clotting time = 1).

Figure 2 is a general diagram of a preferred embodiment of an acousto-mechanical blood biosensor system of the present invention.

Figure 3A and 3B show governing equations and a deformation wave diagram of a TSM transducer exposed on one side to water loading. Figure 3A shows a sensor operating at the fundamental frequency. Figure 3B shows a sensor operating at the fundamental frequency and higher harmonics.

Figure 4 provides a schematic representation of the acoustic blood analyzer sensing process.

Figure 5A and 5B provide a schematic model of a piezoelectric sensor with two acoustic ports and one electric port (Figure 5A), and its electric equivalent circuit representation (Figure 5B).

Detailed Description of the Invention

Clinical hemostasis tests are crucial to millions of patients, who are medicated with anticoagulants and antiplatelet agents. Approximately 12 million patients who undergo surgical procedures require intraoperative or postoperative anticoagulation to reduce risks of thrombosis or stroke. Another 1.5 million patients with atrial fibrillation or prosthetic heart valves take oral anticoagulants to prevent thrombosis and stroke. While these drugs are beneficial, antithrombotic agents can also

cause excessive bleeding (Levine, M. N. and J. Hirsch
(1992). Hemorrhagic Complications of Long-Term
Antithrombotic Treatment. *Thrombosis in Cardiovascular
Disorders*. V. Fuster and M. Verstraete. Philadelphia,

5 Saunders: 515-522), and tests of clotting time are required
to determine proper dosing. Most of these tests are
performed in a clinical lab, rather than at the point of
care or at a patient's home ("self-test"). Patients
undergoing cardiopulmonary bypass or receiving artificial

10 blood pump implants are especially difficult to manage with
traditional clotting tests (Gravlee et al. Ann Thorac Surg
1994 58(1): 216-21). The ideal hemostasis assay for such
patients, the Thromboelastograph (TEG) (Shore-Lesserson et
al. Anesth Analg 1999 88(2): 312-9) measures time and clot

15 mechanical stiffness. However, TEG is difficult to use and
not suitable for self-testing. Platelet function assays,
that serve to differentiate platelet related bleeding
problems from coagulation defects, are also costly and
require specialized technician training.

20   The present invention provides an acousto-mechanical
blood analyzer, also referred to herein as an acoustic blood
analyzer or ABA, for near patient/self testing which
provides accurate and timely platelet-function testing as
well as analysis of the coagulation status of blood and

25 other blood properties.

By the term "blood property" or "blood properties" as
used herein it is meant to include physical, biological
and/or biochemical characteristics of a blood sample.
Examples include, but are in no way limited to, density,

30 elasticity, viscosity, clot stiffness, platelet
concentration, platelet activation, platelet receptor
activities, GPIIb/IIIa function, GPIb function, GPIa/IIa
function, blood hemostatic factor concentration, bleeding

time, activated clotting time, activated partial thromboplastin time, prothrombin time, thrombin time, Fibrinogen, factor VIII deficiency, von Willebrand factor, tissue factor, specific drug concentration, or therapeutic
5   effects of anticoagulation and antiplatelet or thrombin inhibitor drug activities. Further, by blood properties it is meant to be inclusive of characteristics which can be determined by measuring interactions of native or intrinsic components of a blood sample such as, but not limited to
10   cells, proteins, DNAs, or enzymes in the blood or derived from the blood, as well as interactions of extrinsic or foreign components such as, but not limited to, drugs, viruses or bacteria in the blood or derived from the blood.

      In simplest form, the acoustic blood analyzer comprises
15   a transducer section of one or more acoustic sensors, preferably piezoelectric, electrostrictive, magnetostrictive, acoustooptic or thermo(piro) acoustic biosensors or a combination thereof.

      Of these acoustic sensors useful in the present
20   invention, electrostrictive, acoustooptic, and piezoelectric wave transducer modalities have emerged as some of the more promising biosensor technologies. Biosensor development is in large part dependent upon technologies primarily developed for other (e.g. industrial, aerospace, military)
25   purposes. Silicon-based microfabrication (IC) and micromechanical (MEMS) techniques have been successfully applied in fabrication of wide range of miniature electrochemical biosensors. Similarly, progress in optically-based biosensors has its roots in fiber optics and
30   devices designed for fiberoptic communication. In particular, piezoelectric biosensors are the benefactors of decades-long growth in RF telecommunication technologies. Other types of biosensors based on calorimetric or

thermo(piro) acoustic, and magnetic magnetostrictive, techniques, among others, have benefited immensely from modern IC and MEMS research and development. Among all these innovative technologies, piezoelectric techniques offer the

5   broadest range of sensing mechanisms, with low cost and high reliability and thus are preferred at this time for use in the present invention.

In general, however, these acousto-mechanical sensors function by utilizing different types of acoustic waves to

10   "interrogate" the entity being measured, i.e., the measurand. By the term "interrogate" or "interrogates" it is meant that the acoustic sensor generates waves that penetrate the blood sample over some controlled penetration distance or depth into the sample, as measured from the

15   sensor-sample interface (as illustrated in figures 1A and 3). The physical properties of the interrogated sample residing within the wave penetration distance will isolate specific blood sample features and give rise to characteristic signatures as detected by the ABA sensor. By

20   exciting the sensor over a broad range of frequencies, ranging from a single KHz to several GHz, the sensor interrogates the sample over a range of distances from nanometers to centimeters from a surface of the acoustic sensor thus providing multiple measurements for a single

25   blood sample. The acoustic waves can be generated and received by a variety of means, including piezoelectric, magnetostrictive, magnetostrictive, acoustooptic and thermo(piro) acoustic (Lec R.M., and Lewin P.A., Proceedings of 20th Annual IEEE Engineering in Medicine and Biology

30   Society, 1998.20(6): p2785-2790) techniques as well as electrostrictive techniques. Currently, from an engineering point of view, piezoelectric transducer technology offers the most efficient, inexpensive and integrated-circuit (IC)

compatible way of generating of acoustic waves. Further, there are many available materials for manufacturing piezoelectric transducers including crystals, composites and hybrid structures, which provide a wide range of possible

5   sensing material design options.

Piezoelectric transducers deliver mechanical forces to a biological medium usually in the form of progressive or standing acoustic waves. Several different types of acoustic waves can be used for the purposes of sensing

10   biological phenomena. Knowledge of their properties is important for the selection of the optimal acoustic wave for a given biomeasurand. Acoustic waves can be considered a source of distributed force acting on a medium. The resultant deformation force acting on the medium can give

15   rise to compression, torsional, or shear imposed forces, or a combination thereof. The type of the imposed deformation accompanying the wave is important because it determines the resultant acoustic sensing processes. For example, compressional deformation is associated with structural

20   relaxational processes of the medium, while shear is associated with the medium viscoelastic properties, making it sensitive to different molecular processes (Matheson, A.J. "Molecular Acoustic", Wiley-Interscience, New York, 1971; Bathia, A.B "Ultrasonics Absorption", Clarendon,

25   London (1967)). Compressional deformations are easily transmitted through any gaseous, liquid or solid media. Shear deformation, on the other hand, propagates only through solids, and penetrates only to a limited depth into liquid and gases. This latter feature is particularly

30   advantageous, because it makes shear waves sensitive to numerous interfacial phenomena. The shear mode is therefore utilized by many acoustic wave biosensors. The wave penetration depth, which depends on the frequency of the

wave and the density and viscoelastic properties of the medium, ranges from microns to nanometers. Consequently, the required sample volumes for sensing are small, and the sensitivity of the sensor is relatively high. In addition to

5   mechanical phenomena, acoustic wave sensors can sense electrical properties of a medium. Electric field probing of a medium is either generated by acoustic wave displacement (via piezoelectric effect) or is transduced by the sensor electrode structure. Electrical parameters measurable with

10 the acoustic sensors include, but are not limited to, transfer function, frequency, amplitude, phase and quality factor. Thus, acoustic wave sensing mechanisms are robust, capable of measuring the changes in, for example, mass/density, elastic modulus, viscosity, electrical

15 conductivity and dielectric constant.

Acoustic waves usually are generated by an electric section means such as an AC voltage applied to the acoustic sensor or sensors attached to the surface of, for example a piezoelectric substrate, of the transducer section. These

20 propagated waves can be classified as bulk or surface generated waves. For piezoelectric sensors, the bulk generated waves are usually excited by metalized bulk piezoelectric elements such as disks or rods, whereas the surface generated acoustic waves are excited by an

25 interdigital system of metallic electrodes (IDT) placed on the surface of piezoelectric materials. The electrodes are used to connect the transducer with electronic circuitry for the excitation and/or reception of acoustic waves. Two of the most common configurations of piezoelectric transducers

30 are the thin metalized disk and the interdigital transducer (IDT). The thin metalized disk generates bulk waves, which excite Thickness Shear Modes (TSM). The interdigital transducer (IDT) excites Surface Rayleigh Wave (SRW),

Surface Transverse Wave (STW), Shear-Horizontal Acoustic
Plate Mode (SH-APM), and Flexural Plate Wave (FPW). The
STWs form a large family of waves including Shear Horizontal
SAW (SH-SAW), Surface Skimming Bulk Wave (SSBW), and Love

5   wave modes (Josse et al., On The Mass Sensitivity of
Acoustic-Plate-Mode sensors, Sensors and Actuators A,
vol.53, 1996, pp.243-248).

In the acoustic blood analyzer of the present
invention, the transducer section may comprise a single

10   acoustic sensor, two acoustic sensors, particularly for
embodiments wherein measurements are compared to reference
sample, or an array of acoustic sensors comprising either a
single sensor type selected from piezoelectric,
electrostrictive, magnetostrictive, acoustooptic or

15   thermo(piro) acoustic sensors or a combination thereof.
Arrays may also be divided into a sample area and a
reference area for embodiments wherein measurements are
compared to a reference. Use of an array of sensors in the
transducer section improves ABA performance and allows for

20   simultaneous detection of several blood properties.
Preferably, the acoustic sensor or sensors of the transducer
section is designed to generate a plurality of propagated
waves consisting of either pure shear wave, combinations of
shear waves, or a combination of compressional

25   (longitudinal) and shear waves. Torsional waves can also be
measured. Various geometrical configurations of the
transducer section can be used including, but not limited
to, plates, disks, cylinders, tubes, rods, and bars that can
be made to operate individually or in combinations such as

30   in multi-fork structures and multiplate-arrangements.

Piezoelectric nanobiosensors have broad potential to
improve hemostasis testing. As shown in Figure 1A, a shear
mode sensor applies shear deformation to a blood sample, to

a nano- or micro-scale depth controlled by sensor frequency.
As the blood coagulates on the sensor, its mechanical
properties (elasticity and viscosity) change, resulting in
measurable changes in the sensor natural frequency and power

5   attenuation. Because the sensor measures changes in clot
mechanics, it has the diagnostic advantages of the TEG.
When operated at high frequency, the sensor is sensitive to
nanoscale cellular and subcellular processes, such as
platelet adhesion (Lec, R. M. (2001). *Piezoelectric*

10   *Biosensors: Recent Advances and Medical Applications.* IEEE
International Frequency Control Symposium, Seattle, WA,
IEEE). These biosensors for the acoustic blood analyzer
operate in shear wave mode (1 MHz - 50MHz) and combined
shear/compression mode (100-500 kHz).

15   Piezoelectric materials such as quartz crystals, SiO_2,
lithium niobiate ($LiNbO_3$), lithium tantalate ($LiTaO_3$),
langasites and piezoelectric ceramics and electrically
active polymers can be used to make acousto-mechanical
transducers. These materials exhibit a rich spectrum of

20   electro-acoustic properties such as temperature and stress
compensation and strong piezoelectric properties. These
materials also have outstanding mechanical properties, are
chemically stable, and operate at elevated temperatures.
          In a preferred embodiment, an array of high frequency

25   sensors, preferably fabricated of AT-cut quartz crystal, and
low frequency sensors, preferably made with PZT-5A
piezoelectric ceramic are used. Also preferred is an
embodiment, wherein the excitation section means is capable
of exciting the acoustic sensors over a broad range of

30   frequencies ranging from a single KHz to several GHz at
discrete frequencies or simultaneously at all frequencies.
In embodiments where the electronic means has the capability
of excitation of the acoustic sensor at discrete

frequencies, these discrete frequencies may comprise resonant, antiresonant, harmonic and/or anharmonic frequencies. With this frequency range the sensors are able to interrogate clot properties at depths ranging from

5   hundreds of nanometers to tens of microns. The sensor can monitor clotting in static or flowing blood.

In static clotting assays, a blood sample is placed on the surface of the sensor. The sensor resonant frequency, attenuation, and phase is monitored using a Network Analyzer

10   for a selected group of harmonics as a function of time. As a result the kinetics and the signatures of targeted processes can be determined. It is expected that the signatures will be unique for a given targeted blood process and will provide important biochemical information.

15   In a preferred embodiment, the acoustic sensor further comprises a bioactive surface or coating which promotes specific cell-sensor and blood sensor interactions. Such bioactive surfaces or coatings are selected in accordance with the blood property to be measured to promote specific

20   interactions with native or intrinsic blood components including, but not limited to, cells, proteins, DNA, or enzymes or foreign or extrinsic blood components including but not limited to, drugs, viruses, or bacteria specific to the blood property being measured. Dedicated

25   (bio)functionalized surfaces of acoustic sensors of the transducer section improves ABA performance and allows for detection of several blood properties

A preferred design for an acousto-mechanical blood analyzer of the present invention is depicted in Figure 2

30   and further comprises a fluidic section, an electronic section means and a packaging section or housing for integrating the transducer section with fluidic section and the electrical section means. Also preferred are analyzers

of the present invention comprising an integrated blood
sampling means.

The fluidic section of the acoustic blood analyzer is
an interconnected two-dimensional or three-dimensional
5   structure formed by the transducers that employs a
microfluidic system to deliver and distribute blood samples
to the sensor testing areas of the transducer section. The
sensor testing areas of the transducer section are the
regions close to the surface of the sensors. These areas
10 are designed in various geometries such as cylinders or
cones which are appropriate for confining blood samples at
the transducer surface and maintaining its measurement
integrity. The microfluidic system of the analyzer of the
present invention utilizes microchannels, pumping,
15 capillarity and/or mixing for handling blood and for
creating programmable blood flow conditions. Preferably the
microfluidic system or circuit utilizes programmable
acoustic waves to propel the blood sample along the surface
of the biosensors. Thus, the fluidic section means of the
20 acoustic blood analyzer of the present invention has the
capability of delivering blood to the acoustic transducers
utilizing forces inclusive or, but not limited to,
electrical, mechanical and thermal forces as well as
gravitational, electrophoresis, capillary and/or magnetic
25 forces. In addition to moving the blood sample, the
fluidics section of the present invention may also mix
and/or heat the blood sample to a selected temperature.

The electronic section means, also referred to as a
signal processing means, of the analyzer excites the sensors
30 of the transducer section into appropriate vibrational modes
and detects changes in the operational parameters of the
transducers. Vibrational modes useful for characterizing
physical, biochemical and/or biological blood properties

include, but are not limited to, shear, torsional, and compressional vibrational modes, as well as combinations thereof. The detected changes include variations in the transfer function, the resonant frequency, the resonant

5   amplitude, the phase and the quality factor. Measured changes are processed, related to the targeted blood property, and displayed. Accordingly, the electronic section means comprises a system of accompanying electrical oscillatory circuits in which resonant transducer structures

10 control their frequency, phase and the amplitude. The electronic section means used in the acoustic analyzer must be capable of excitation of the one or more acoustic sensors of the transducer section over a frequency range of a single KHz to several GHz. Excitation may be performed at discrete

15 frequencies or simultaneously at all frequencies. When excitation is performed at discrete frequencies, these frequencies may comprise resonant, antiresonant, harmonic and/or anharmonic frequencies of the first and/or higher orders. Exemplary electronic measurement systems useful in

20 the present invention include, but are not limited to, oscillators, phase-lock loops, vector voltmeters, sing-around circuits, network analyzers and spectrum analyzers.

The packaging section of the analyzer comprises a housing that provides mechanical and functional integrity to

25 the transducer section, the fluidic section and the signal processing means. The packing section also provides an interface for the analyzer with analytical laboratory systems as well as computer based data processing, storage and display systems.

30 Some embodiments of the analyzer of the present invention may further comprise a blood sampling means integrated into the analyzer for obtaining a drop of blood from a subject. Any well-known blood collection unit can be

integrated into the analyzer. Examples include, but are in no way limited to a needle, a MEMS based micro-pricking system, or a finger stick/prick collection means.

The fundamental principles of operation of the acoustic sensor or sensors used in the acoustic blood analyzer of the present invention can be inferred by following its active sensing pathway. Blood is introduced to the acoustic sensor or sensors using a blood sample collection device either separate from, or more preferably integrated in stand-alone systems of the present invention or by bringing the acoustic blood analyzer to the patient, as with other "near-patient" or indwelling biosensor probes. Accordingly, the present invention also relates to devices for measuring blood properties in the body of a subject comprising the acoustic blood analyzer of the present invention integrated into a catheter to be inserted in the body of the subject. Various configurations for stand-alone and integrated ABA systems can be used including, but not limited to, a flow-through system, a dipstick, a smart syringe or integrated in an extracorporeal blood conduit. The blood is preferably passed through a preprocessing section of the microfluidic mechanism such as a selective membrane interface, which performs an initial filtering of blood constituents. Thereafter, the blood sample is exposed to a sensing element, preferably a biologically active substance such as collagen or thromboplastin, which is selectively responsive to a measurand of interest such as platelets, blood cells, or a selected protein. When the biomeasurand interacts with the sensing element, microscopic physical, chemical, and/or biochemical changes are produced. These microscopic changes cause the macroscopic physical changes in the biosensing element, which are converted by the acousto-mechanical transducer into a measurable electric signal output. The

electric signal is conditioned, processed and displayed. In particular, the processing module can be made to recognize such important sensor features as self-calibration, self-diagnostic and advanced pattern recognition analyses. All of these functional design elements can be encapsulated in the packaging unit that provides measurement integrity to the device.

The blood acousto-mechanical analyzer of the present invention can be used to measure and characterize multiple blood mechanical and acoustic properties excited at fundamental and harmonic frequencies. This allows monitoring the blood properties at different probing depths of the given blood sample ranging from distances of nanometers to centimeters from the surface of the acoustic sensors of the analyzer.

In studies with the analyzer of the present, the whole blood samples exhibited responses which were singular to the frequencies used, thus providing specific signatures corresponding to different blood interfacial processes. This feature of the acoustic blood analyzer sensing technique is unique and very important because it provides telling and interpretation-rich one-dimensional mapping of the interfacial processes and mechanical properties of the media adjacent to the sensor surface. Schematically, the cross-sectional sensing interface is depicted in Figure 3, where a graphical representation of a distribution of shear mechanical displacement generated by a acousto-mechanical shear transducer immersed in water is given when the transducer operates at fundamental (Figure 3a) and harmonic (Figure 3b) frequencies. As a general rule, acoustic transducers can effectively "slice" a biological interface at different depths (Figure 3b), hence providing important spatial information.

Using the analyzer of the present invention properties including but not limited to the density, viscosity, elasticity, electric conductivity and dielectric constant of blood can be assessed by the blood-sensing element interface

5   undergoing changes (during the measurement process), which in turn modify the acoustic field quantities of the acoustic wave transducer. Macroscale changes in elastic and visco-elastic properties of blood have been used successfully to monitor and characterize blood clotting in the

10   thromboelastograph (TEG) and the clot rheometer. The acousto-mechanical wave transducer, which consists of an acoustic sensor, preferably a piezoelectric element with an array of metal electrodes, acts as an energy converter which transducts biomeasurands into output electric signal. For

15   example, in the blood piezoelectric sensors a collagen thin film can be deposited as the bioactive agent on the surface of the transducer. When the target measurand, such as platelets, interact with the sensing collagen coated surface, the resultant elasticity, density and viscosity of

20   the film can vary, modifying the acoustic parameters of the sensor and thereby leading to the changes in the output sensor signal. Schematically, the sensing sequence process is depicted in Figure 4.

Since the boundary conditions or the topography of a

25   sensor surface are particularly important and influence sensor response, it is preferred that the acoustic blood analyzer surface be functionalized with programmable properties in order to achieve the requested specificity and sensitivity of the sensors.

30   Various biofunctionalization strategies for increasing sensor specificity and sensitivity through a bioactive agent on the surface of biosensor can be used.

For example, in one embodiment the sensor is coated with an antibody specific to an unactivated or activated coagulation factor such as factors II, V, VII, VIII, IX, X, IX, or an activated coagulation enzyme complex such as
5   tenase or prothrombinase, or to von Willebrand factor. Such coating will increase the sensor sensitivity to the target factor when the sample is interrogated at the appropriate molecular length scale.

In another embodiment, the sensor surface may coated
10   with a specific activator of a coagulation factor, such as tissue factor, the natural extrinsic activator or coagulation factor VII (see example 5).

In another embodiment, the sensor may be coated with fibrillar collagen to increase sensor sensitivity to
15   platelet adhesion and von Willebrand factor adsorption. In the platelet adhesion assay configuration, the blood flow shear rate at the sensor surface may be controlled by the microfluidic circuit design to preferentially measure either function of platelet GPIa/IIa at lower shear rates, or
20   function of GPIb at high shear rates.

In another embodiment, the sensor surface may be coated with fibrinogen to increase sensitivity to platelet activation and GPIIb/IIIa function. Further, platelet activation may be stimulated by addition of agonists
25   including but not limited to ADP, epinephrine, a stable thromboxane analog, or platelet activating factor.

In another embodiment, the sensor surface may be coated with a specific antithrombin or thrombin inhibitor such as PPACK or hirudin to increase sensitivity to thrombin
30   generation at the sensor surface.

In another embodiment, the sensor surface may be coated with a substrate for thrombin or another coagulation cascade activated factor, which may be digested by the factor

leading to a sensor attenuation decrease at the time of factor activation.

In another embodiment, the sensor may be coated with phospholipids such as, but not limited to, phosphoryl

5   choline and phosphoryl serine to provide a substrate for coagulation complex (tenase and prothrombinase) assembly and to enhance sensor sensitivity to tenase and prothrombinase.

In yet another embodiment, the sensor is coated with an antibody capable of detecting a selected intrinsic or

10   extrinsic component in the blood. For example, the sensor can be coated with an antibody to a cell specific antigen such as an antigen on the surface of leukemia cells.

An important feature of a piezoelectric sensor is that it can directly measure mechanical/acoustic properties of

15   liquid medium by electrical means. In piezoelectric materials, the electro-acoustic transduction takes place within the material and is almost instantaneous. Therefore, any changes in the liquid are quickly (msec) detected by the sensor. A knowledge of the nature of this sensing process

20   is critical for proper interpretation of the sensor response. When one applies a voltage to the sensor, the sensor creates a shear deformation/displacement at the interface. This deformation is transmitted, via the boundary conditions, to the liquid media such that a stress field is

25   generated at the interface. The parameter that characterizes (captures) that process is the mechanical impedance of the solid-liquid interface. Effectively, the piezoelectric sensor can be modeled as a microdevice that transforms mechanical impedance into electrical impedance as

30   schematically shown in Figure 5A. The electro-mechanical impedance has a resonant property that can be represented in the form of an electric circuit given in Figure 5B. The components of that circuit are functions of the

piezoelectric material (C_o, L_1, C_1 and R_1) and the measured media, (L2 and R2 are related to liquid medium, and L3 describes mass deposition at the surface). The mathematical expressions are a dependent function of assumed (or

5 measured) properties of the liquid medium. Thus, blood properties are measured by correlating an electrical property of the acoustic sensor utilizing a mathematical solution to the coupled Newton, Maxwell and blood constitutive equations, and the boundary conditions

10 representing an actual sensing process, and whose solutions have a form of mathematical analytical and/or numerical expressions representing the acoustic sensor electrical parameters such as the transfer function, impedance, admittance, quality factor, resonant frequency and its

15 difference, and phase. For example, Newtonian liquids media, $\omega_s L_2 = R_2 = A \cdot (\rho\eta)^{0.5}$. For viscoelastic media these expressions are more complex. In addition, mass (solid phase) accumulation at the interface can be represented as an another inertial (inductive) component, $L_3 = B \cdot (\rho_{in})$,

20 where A and B are the sensor constants, and ω_s, is the resonant frequency of the sensor.

In general, different media and processes will be represented by different structures of the electric equivalent circuit representation and by different

25 functional relationships between mechanical parameters and the corresponding electric components. It is important to note that such a circuit representation provides a powerful tool for quantitative interpretation of interfacial phenomena. For example, considering a sensor whose resonant

30 frequency is $\omega_{s1} = (L_1 C_1)^{-0.5}$ and whose losses are determined by R_1, when immersed in liquid, it can be represented by adding new circuit elements (L_2 and R_2) to the electrical side of the sensor. The resulting resonant frequency and the

associated viscous losses are made manifest by the decrease
in resonant frequency ω_{s2} $[(L_1+L_2)C_1]^{-0.5}$ and the increase in
equivalent resistance $R_{tot}= R_1 +R_2$. Now, if mass (e.g. a few
platelets) accumulates at the interface, the frequency will

5   change again because the total inductance increases $L_{tot}= L_1 +$
$L_2 + L_3$; however, the "resistive" losses will remain nearly
constant. Moreover, if the mass increases and the solid
layer thickens (e.g. more platelets), then some viscous
losses will start to emerge, and we will observe additional

10 R_3 losses. If sample conditions are such that the platelets
start to acquire structural stiffness, then the viscous
losses will be lower, the frequency will increase, and
overall losses will decrease. Importantly, observing the
evolution of the sensor response characteristics as a

15 function of time will enable the means to monitor subtle
interfacial processes, providing novel insight and
interpretation of coagulation biology.

Acoustic blood sensors of the present invention can be
fabricated using standard photolithographic and MEMS

20 technology. Photolithography allows fabrication of complex
sensor electrode patterns, and production of multiple
sensors from a single substrate. Micromachining technology
(MEMS) is used for manufacturing ABA sensors operating at
high fundamental frequencies above 100 MHz.   The

25 micromachining technology, which combines the
photolithographic process and plasma or chemical etching
techniques, enables development of complicated mechanical
and electrical sensor microstructures from a single
substrate.   The biological sensing interface is preferably

30 fabricated using adsorption, evaporation, spinning, soft
lithography patterning and self-assembly techniques such as
described by Andersson, L.I. (Journal of Chromatography B,
2000, 745:3-13) and Kane et al. (Biomaterials, 1999.20:2363-

2376). In embodiments of mass scale biosensor manufacturing process steps for a biological film attachment are integrated with the piezoelectric and electronic fabrication steps.

5   Use of the acoustic blood analyzer enables a broad range of medical services at a patient's home using a variety of systems that employ a personal computer and web-based guided access to the Internet. One can envision a dedicated home-based analytical diagnostic system interfaced

10   with a computer that would monitor and store medical data, spanning the lifetime of the patient. In cases of this kind, specialized application software would be able to recognize impending health-related problems and alert the user of her or his health conditions. Small and sensitive

15   biosensors would also assist the general population and health-care providers in monitoring a variety of "environmental conditions" critical to the health and well being of a given patient population.

The acoustic blood analyzer of the present invention is

20   particularly useful in measuring blood coagulation, specific coagulation cascades steps, including activation of plasma factors VII, IX, XI, VIII, V, X, and II (thrombin), as well platelet functions. The acoustic blood analyzer is therefore useful in identifying, diagnosing and monitoring

25   subjects at high risk for blood clots and excessive bleeding. For purposes of monitoring subjects, sampling and analysis may be performed on a periodic basis depending upon the condition and the severity of the condition in the subject. For example, for subject receiving anti-coagulant

30   therapy following surgery, monitoring may be performed daily or every other day.

In general, to use the analyzer a blood sample is first prepared, for example, by taking the blood sample directly

from a patient and/or mixing it with a specific reagent such as a drug or a functional chemical, or group of reagents. The prepared blood sample is then delivered to the surface of the acoustic sensor or sensors of the acoustic blood

5   analyzer. In some embodiments, a reference fluid such as air, buffer, or a control blood sample is also placed on the surface of a second acoustic sensor of the analyzer and used as a reference and/or as a standard measurement signal. Electrical changes in the characteristic fundamental and

10   harmonic resonant frequencies, phase, amplitude, impedance, admittance or trans-impedance/admittance of the acoustic sensor or sensors in the presence of the blood sample, and when used the reference fluid, are then monitored. The resulting signal is processed to correlate the electrical

15   parameters of the acoustic sensor, or both the acoustic and the reference sensors, with the appropriate parameters characterizing blood properties including, but not limited to coagulation, platelet functions, and other processes. Quality assurance is implemented by minimizing the influence

20   of the ambient conditions such as temperature fluctuation, blood flow, blood background signals, etc. on the final blood testing results. Following analysis, the blood sample is disposed of in an appropriate waste container. Results of the analysis are presented in a readable form using

25   display screen, printer, computer, etc.

        The acoustic blood analyzer of the present invention is also useful in testing the blood-compatibility of natural and synthetic materials as well as the impact of drugs on blood properties. Thus, the acoustic blood analyzer of the

30   present invention can be used in the drug discovery process of blood related medicines.

        The following nonlimiting examples are provided to further illustrate the present invention.

EXAMPLES

Example 1:   Blood Samples

In experiments where active platelets are required, porcine blood is obtained from a local slaughterhouse
5   (Hatfield Pork), drawn from a carotid/jugular incision into 3.2% trisodium citrate (1 part to 9 parts blood), and used within 8 hours. Clotting is initiated by recalcification with $CaCl_2$ solution. In some assays, ADP is added to activate platelets, or kaolin is added to directly activate
10   intrinsic coagulation. In other assays, coagulation is delayed by addition of heparin, or clot is weakened by inhibition of platelet contraction using platelet glycoprotein inhibitors or cytochlasin D. For standardized assays, blood analogs (lyophilized blood coagulation test
15   standards) are obtained (Heamocron) and activated with celite. In some assays, fibrinolysis is stimulated using tissue-type plasminogen activator, to evaluate potential for the sensor to detect fibrinolytic tendency.

Example 2: Analysis of Blood Samples via the Acoustic Blood
20   **Analyzer**

The blood acoustic analyzer was used to monitor clotting blood under a variety of conditions. Fresh porcine blood from a carotid/jugular incision was collected into 3.2% citrate (1 part to 9 parts blood) and stored at room
25   temperature in high-density polyethylene containers. Blood was used within 8 hours in all experiments to preserve platelet function and coagulation protein concentrations. In most experiments, clotting was initiated by addition of $CaCl_2$ solution to recalcify the blood, with rapid mixing and
30   immediate application of 200 µL to the sensor surface using a micropipette. Ten mL of the recalcified blood was kept in a 50 mL polypropylene tube, and gently inverted periodically

to monitor clotting. The clotting time was defined as the
time at which the blood sample resisted inversion. In this
system, clotting time was on average 10 minutes for fully
recalcified blood samples. In one experiment, clotting time

5   was delayed by reducing the $CaCl_2$ concentration of
recalcified blood. In a second experiment, clotting time
was delayed by addition of heparin (0.25 U/mL final
concentration) to the blood sample. In some cases, 5 µM ADP
was added following recalcification, to activate platelets

10   and provide procoagulant surface which accelerates
coagulation.

       All experiments were conducted using a 5 MHz
piezoelectric shear mode sensor. The sensor was used in two
configurations. In some cases, blood was monitored directly

15   on the gold sensor surface. In other cases, the sensor was
coated by bovine achilles tendon collagen type I fibers
(Sigma) suspended in acetic acid, incubated on the sensor
for 60 minutes, then rinsed with isotonic saline and allowed
to air dry.

20   For the experiments to compare sensor response to
different blood constituents, platelet-rich plasma was
prepared by centrifugation of 50 mL of whole blood at 300g
for 10 minutes, and gently pipetting 14 mL of supernatant.
Platelet-poor plasma was prepared by centrifuging 14 mL of

25   platelet-rich plasma at 3000g for 30 minutes, and pipetting
9.4 mL of supernatant.

Example 3: Uncoated Sensor

       Clotting blood causes an increase in sensor attenuation
and a decrease in resonant frequencies which was clearly

30   distinguishable from citrate-anticoagulated blood, which
caused a negligible change in resonant frequency, and only a
small rapid increase in attenuation, as shown in Figure 1B.
The dynamics of these changes correlated with mechanical

clotting time as shown in Figures 1C and 1D, demonstrating
that the sensor is suitable for detecting clotting time.
The uncoated sensor also reacted rapidly and strongly with
heparin in heparinized blood samples.

5   **Example 4:   Collagen-coated Sensor**

        The sensor described in example 2 was coated with
bovine achilles tendon collagen type I fibers (Sigma) by
adsorption from acetic acid suspension, incubated on the
sensor for 60 minutes, then rinsed with isotonic saline and
10   allowed to air dry. The collagen coating increased sensor
sensitivity to early hemostatic events associated with
platelet function. These early clotting events may be
mediated by platelet activation, as demonstrated by the
increased rate of attenuation change when ADP is added to
15   activate platelets. The sensor response amplitude differs
between whole blood, platelet-rich plasma, and platelet-poor
plasma clots. This response may be indicative of specific
interactions with red blood cells, or a sensitivity to clot
stiffness and viscosity.

20   **Example 5:   Tissue Factor-Coated Sensor**

        A 10 MHz piezoelectric shear mode biosensor was coated
by tissue thromboplastin (Fisher Diagnostics) by adsorption
to the sensor surface from diluted solution in sodium
carbonate buffer for 60 minutes, followed by rinsing with
25   phosphate-buffered saline. 200 µl samples of platelet-poor
plasma were placed on the sensor surface with buffer
(control sample) or CaCl2 (recalcified sample) added and
mixed. The sensor operated at the 5th harmonic (50 MHz) was
able to detect a rapid interaction between the recalcified
30   sample and tissue-factor coated sensor, followed by a slower
attenuation increase that corresponded to the bulk sample
clotting time. These interactions were not observed in the

control sample. The bulk clotting attenuation increase in the recalcified sample was also observed at the fundamental and 3rd harmonic, but the early attenuation increase was greatly reduced at these frequencies.

5   The early interaction is attributed to activation of factor VII by the tissue factor coating on the surface, based on the calcium dependence of this activation event, the specific interaction between factor VII and tissue factor, and the increased sensitivity at higher frequencies

10   corresponding to molecular length scales.